Doctoral (*Ph.D.*) theses

Design of macromolecular colloids-based drug delivery systems

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1. Introduction and main goals

The polymer-based nanostructured drug carrier systems play an increasing role in the fields of biomedicine, nanomedicine, and pharmacy. By using them, we can prolong and increase the efficacy of the drugs; furthermore, it is possible to introduce therapeutic compounds which are significantly hampered by the specific properties of the given active ingredient. (e.g. hydrophilicity, charge, degradation). Due to their biocompatibility and easy handling, the application of various (bio)polymers (e.g. polycaprolactone (PCL), poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA)) and the polysaccharides (e.g. hyaluronic acid, chitosan) has a great potential. By structural modification of these above- mentioned macromolecules (e.g. copolymerization, neutralization, cross-linking, etc.), their hydrophilic feature can be systematically changed, which can facilitate the delivery of active ingredients to the polymer particle. In addition, further advantageous properties (e.g. solubility, increased encapsulation efficiency, formation of core-shell structure, controlled drug release, etc.). can be achieved via the use of complex *e.g.* biopolymer / polysaccharide-based composite particles. Thanks to the wide variety of nanoprecipitation-, emulsion-, double emulsion- and flow-based preparative techniques, nowadays the preparation of drug-containing colloidal particles can be carried out in many ways.

In our research group, the preparation of mostly protein-based drug carrier colloid particles is received special attention for several years, where the successful penetration of neuroactive molecules across the blood-brain barrier is the main goal. I joined to these researches in the autumn of 2017, where, within the framework of a newly won GINOP-2.3.2 tender, my main task was to design new type of, mainly polymer- and polysaccharide-based drug delivery systems via development of reproducible syntheses. We aimed to develop dominantly PLA, PLGA and hyaluronic acid (HyA) macromolecular colloid-based carrier systems, where we planned to study the effect of the hydrophilicity, structure and surface charge of these carrier systems on the encapsulation efficiency of the hydrophilic or hydrophobic, aromatic or only aliphatic groups-contained, charged or neutral model small molecules. We planned to determine what experimental factors influence the size, structure, and morphology of the formed particles. Where possible, we also aimed to study the kinetics of drug release as well.

2. Experimental methods

For the preparation of the stock solutions, the buffers, and the aqueous polymer particles, high purity Milli-Q water (Millipore, MilliQ Integral 3, conductivity at 25 ° C 18.2 MΩ × cm) was used in all cases. The syntheses and the measurements were performed with analytically pure chemicals, without the using of pre-purification. The structural formulas of the corresponding monomer units of the polymers, the stabilizing agents and the (model)drugs, which were used to the preparation of the drug carrier particles, are shown in **Figure 1**.

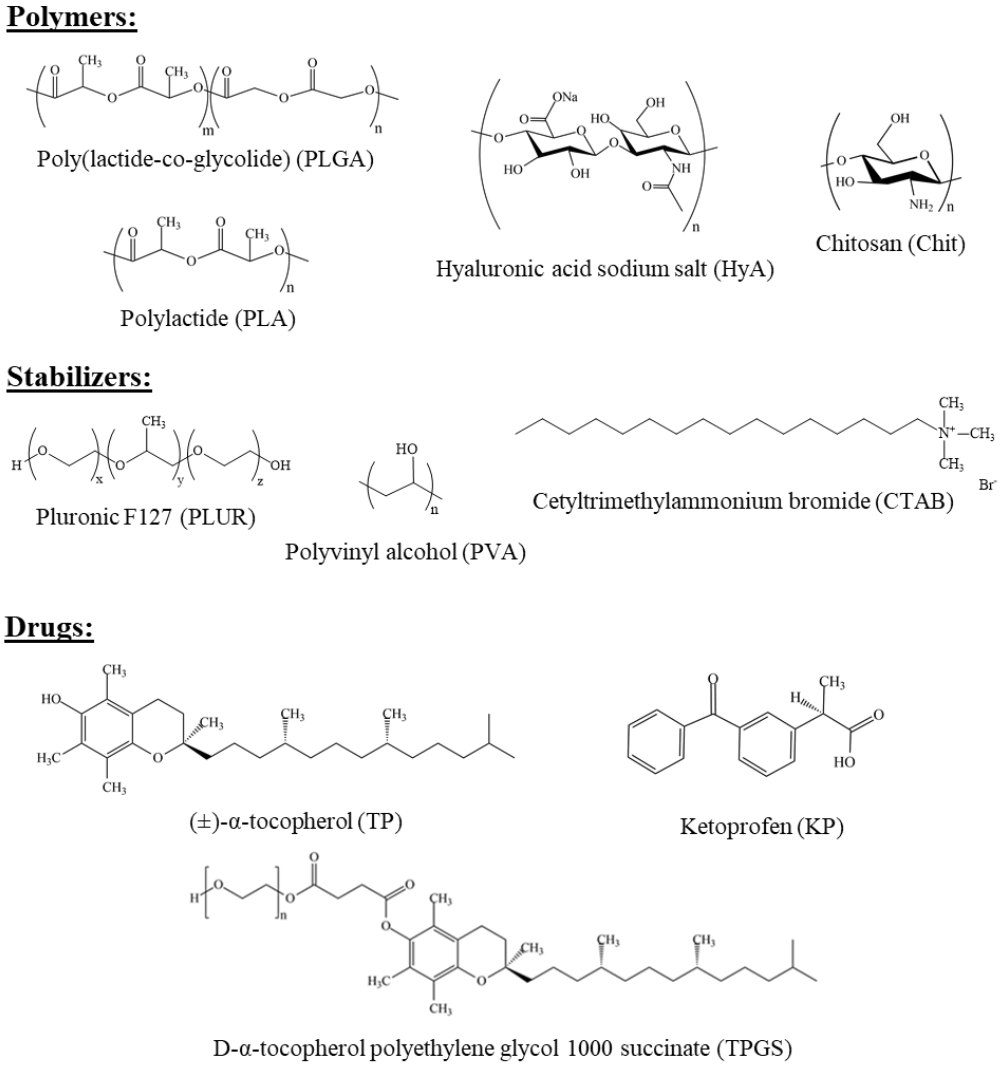

Figure 1. The structural formulas of the corresponding monomer units of the applied polymers, the stabilizing, and the drug molecules

The abbreviated names of the substances/compounds are also shown in **Figure 1**, thus these abbreviated names will be presented for each thesis points. In the case of the PLGA50 /65/75 derivatives, the numbers indicate the proportion of the lactide percentage content in the

polymers. The synthesis of the various modified polymers and the methods of the preparation of the polymer-based particles are detailed in the dissertation.

For studying the size, size distribution, structure and the composition of the polymer-based carrier systems, the following measurement techniques are used (The abbreviated names of measurement techniques (marked in italics) are also given in brackets):

- Fourier-transformed infrared spectroscopy (*FT-IR*; Jasco FT/IR-4700)
- Differential scanning calorimetry (*DSC*, Mettler-Toledo 822e)
- Thermogravimetry (*TG*, Mettler-Toledo TG/SDTA 851^{e})
- Contact angle measurement (KRÜSS FM40Mk2 Easy Drop)
- Charge titration (Mütek PCD-04)
- Isothermal titration calorimetry (*ITC*, MicroCal VP-ITC)
- Turbidimetry (LP2000 Hanna Ins., Ocean Optics USB4000)
- Conductometry (Radelkis OK-114)
- Rheology (Anton Paar MCR 301)
- Dynamic light scattering, molecular weight and ζ-potential measurements (*DLS*, Horiba SZ-100 and Malvern NanoZS)
- Transmission electron microscopy (*TEM*, Jeol JEM-1400plus)
- High resolution transmission electron microscopy (*HR-TEM*, FEI Tecnai G2 20 X-Twin)
- UV-Vis spectrophotometry (Shimadzu UV-1800)
- Tensiometer (KRÜSS K100MK2)
- Freeze-drying (Christ Alpha 1-2 LD)

3. New scientific results

(T1.) The size, the structure, and the stability of the PLA/PLGA-based colloidal drug delivery systems, produced by nanoprecipitation, are jointly affected by the solvent, the stabilizer and the hydrophilicity properties of the carrier/drug, which used for syntheses.

PLA and PLGA polymers with different lactide/glycolide ratios were successfully synthesized by ring-opening polymerization process. The wetting properties, which characterized by contact angle properties, were in good agreement with the contact angle values of the same commercially available polymers. Furthermore, we determined that, the hydrophilicity of the copolymers is systematically increased with increasing glycolide content (PLA: 74.55 ± 0.82°, PLGA75: 70.50 ± 0.25°, PLGA65: 68.18 ± 0.61°). Based on the determination of the average molecular weights by light scattering experiments and the precipitation titration curves, our synthesized PLA / PLGA polymers show a relatively low molecular weight and narrow molecular weight distribution.

- We obtained that, the hydrodynamic diameter of our synthetized different hydrophilicity PLA/PLGA polymer-based particles prepared by nanoprecipitation method, can be controlled by the material quality (*e.g.* boiling point, difference between the surface tensions) of the solvents (1,4-dioxane, acetone) and the chemical structure (molecular weight, charge) of the stabilizer molecules (PLUR, PVA, CTAB) as well. The DLS measurements and the recorded TEM images pointed out that the application of the acetone, with lower surface tension and boiling point than 1,4-dioxane, resulted in significantly smaller (with 10–70 nm less) particles. For the stabilizer molecules, the largest hydrodynamic diameter was obtained by positively charged CTAB (e.g., ~261 nm (PLGA75)), while the smallest was achieved using PLUR (e.g., ~180 nm (PLGA75)), which confirmed, the size of the drug carrier particles was regulable with the appropriate selection of the stabilizer. In addition, the hydrophilicity property of polymers also has a significant influence on the hydrodynamic diameter.
- Based on the determination of the encapsulation capability of the PLA/PLGA macromolecular colloids, we stated that, in case of the different hydrophilicity compounds (TP, KP and TPGS), the formation of the core-shell structured drug carrier systems is become possible by the decreasing of the hydrophilicity of the molecules.

(T2.) The formation of well-defined core-shell-structured tocopherol (TP)-containing PLA/PLGA particles with sufficient colloid stability can only be achieved by setting specific carrier / drug / stabilizer concentration values. The amount of the encapsulated drug and its dissolution can be controlled by the hydrophilicity properties of the carrier polymer.

- By electron microscopic images, we showed, that the formation of the core-shell structure of the TP-loaded PLA particles is become unfavourable with the decreasing of the component concentrations. By using optimized concentration parameters ($c_{PLA/PLGA}$= 10 mg/mL és c_{TP}= 2.5 mg/mL (1 mL acetone phase), c_{PLUR}= 0.1 mg/mL (10 mL water phase)), depending of the lactide/glycolide, the average hydrodynamic diameter of d = 200 - 225 nm can be achieved.

- As the glycolide ratio into the PLA/PLGA increases, the encapsulation efficiency of the TP can be increased. Examining the drug content of the colloidal particles, it was confirmed that the highest encapsulation efficiency was observed for PLGA65 (PLA: 69 %, PLGA75: 76 %, PLGA65: 88 %).

- Based on the *in vitro* dissolution curves, it was determined that the increasing hydrophilicity and drug loading of the polymers is resulted the increasing of drug retention (PLA: 35.0 %; PLGA75: 28.3 %, PLGA65: 19.8 %). Of the kinetic models, which we fitted to the dissolution data by nonlinear regression, the best fit is given by the Weibull and Korsmeyer-Peppas equations ($R_2 \approx 0.99$). The successfully defined kinetic parameters confirm the mainly diffusion-controlled nature of the dissolution processes.

(T3.) We firstly prepared core-shell nanostructured TP-containing PLGA50 colloidal particles by continuous flow chemistry technique.

- In a microreactor (μ-mixer cell) -equipped continuous flow (Asia Flow) apparatus, we successfully produced TP-free and TP-loaded PLGA50 colloid size particles. By the optimizing of the synthesis parameters, we determined, compared to the nanoprecipitation method (~160 nm), smaller hydrodynamic diameter core-shell nanostructured particles can be produced by this method (~135 nm).

- We pointed out that, in contrast to the classical and frequently used nanoprecipitation method, this flow chemical technique can be used to produce PLGA-based TP- or similar structure and solubility molecules-loaded colloid particles in cost-effectively, shorter time and

larger quantities. We confirmed, that similar values can be obtained for the encapsulation efficiency (~67.1 % (nanoprecipitation), ~71.5 % (flow method)).

(T4.) Cross-linked and CTAB-modified partially hydrophobized hyaluronic acid nanoparticles are suitable for encapsulation of ketoprofen (KP) having hydrophobic character. The dissolution of the KP can be controlled by increasing the degree of the cross-linking and the HyA/CTAB ratio.

- We successfully demonstrated by dynamic light scattering measurements and TEM recordings, the partial (50-75 % cl-HyA) and complete (100 % cl-HyA) diamine-cross-linked hyaluronic acid having 200-500 kDa molecular weight can be efficiently used to produce purely hyaluronic acid-based colloidal particles. It was further determined that the hydrodynamic diameter of the formed particles can be increased from d ~ 45 nm (50 % cl-HyA) to d ~ 110 nm (100 % cl-HyA) by increasing the degree of cross-linking. The highest colloidal stability was found for the 100 % cross-linked derivative (ζ ~ -23.6 mV).
- In addition to the formation of the cross-links, hyaluronic acid/ surfactant complex nanoparticles were successfully prepared by cationic surfactant (CTAB). Conductivity, isothermal titration microcalorimetry, rotational viscosity and ζ-potential measurements quantitatively characterized the interaction between the macromolecule and the surfactant, where we pointed out that one negative charge of HyA monomer unit can be compensated by nearly one CTAB molecule. The formation of colloidal particles is the favourable until full charge compensation (d ~ 50 nm), but after the charge neutralization, the high degree of the aggregation of the particles can be observed, which is already unfavourable for using as drug delivery system.
- Rheological studies have confirmed the polymer solutions and the hydrogels show Newtonian, pseudoplastic then viscoelastic behaviour with increasing of the HyA concentration (from 0.05 mg/mL to 100 mg/mL). Based on the oscillation measurements of the hydrogels, we showed that the increasing of the concentration, reaching of the 100 mg/mL HyA concentration, the elastic behaviour of the gels is dominant as against of the viscous property. It was further found, at the cross-linked hydrogels by the increasing of the cross-link degree, due to the disintegration of the coherent gel structure, the viscous-, while in case of the increasing of the surfactant concentration at the cationic surfactant neutralized association colloids, the elastic property is become dominant.

- The cross-linked and the HyA / CTAB nanoparticles were successfully used to encapsulate KP molecules, where we determined by the dissolution curves that measurably high drug retention could be achieved with HyA / CTAB nanoparticles. The Korsmeyer-Peppas and Weibull kinetic models, which were shown best fitted to the data, were pointed out, the diffusion-controlled drug release is dominant for all cases of cl-HyA samples, while increasing of the CTAB amount in the HyA / CTAB system, on addition to diffusion control, the effect of erosion processes is become stronger.

(T5.) Using multiple physico-chemical measurement techniques, we successfully characterized the degree of the electrostatic interactions between the hyaluronic acid and chitosan macromolecules having different charge per monomer unit. Based on these information, we optimized the preparation protocols of chitosan-modified hyaluronic acid-based drug delivery particles.

- The interaction of chitosan and hyaluronic acid macromolecules was studied quantitatively. We confirmed that, the degree of the electrostatic interaction between the one positive (Chit) and one negative charge (HyA) monomer units-contained macromolecules, thereby the expected total charge compensation at 1:1 monomer molar ratio, is strongly influenced by the pH of the medium and the degree of the deacetylation of Chit. FT-IR and thermoanalytical measurements have proved that, regardless of the initial Chit:HyA mass ratio, the electrostatically compensated complexes have a constant composition.
- Knowing the interaction between the macromolecules, we successfully prepared electrostatically compensated HyA / Chit, tripolyphosphate (TPP) cross-linked Chit-TPP / HyA, and core-shell structure Chit-TTP_{core} / HyA_{shell} colloidal particles by using different production protocols. Based on the DLS results, we found that the formation of small particles (d = 100 – 300 nm), regardless of the type of the above listed particles, is beneficiary in case of the significant Chit (m_{Chit} / m_{HyA} = 20: 1 to 80: 1) or HyA (m_{Chit} / m_{HyA} = 1: 8 to 1: 2) macromolecule predominance.

4. Publication list

Hungarian Scientific Bibliography (MTMT) identifier: 10067813

Publications related to the scientific topic of the dissertation:

[1] Á. Turcsányi, **N. Varga**, E. Csapó, *Chitosan-modified hyaluronic acid-based nanosized drug carriers*, International Journal of Biological Macromolecules, 148 (2020) 218-225. doi: 10.1016/j.ijbiomac.2020.01.118

IF_{2019} = 5,162

[2] **N. Varga**, Á. Turcsányi, V. Hornok, E. Csapó, *Vitamin E-Loaded PLA- and PLGA-Based Core-Shell Nanoparticles: Synthesis, Structure Optimization and Controlled Drug Release*, Pharmaceutics, 11 (2019) 357. doi: 10.3390/pharmaceutics11070357

IF_{2019} = 4,421

[3] **N. Varga**, V. Hornok, L. Janovák, I. Dékány, E. Csapó, *The effect of synthesis conditions and tunable hydrophilicity on the drug encapsulation capability of PLA and PLGA nanoparticles,* Colloids and Surfaces B: Biointefaces, 176 (2019) 212-218. doi: 10.1016/j.colsurfb.2019.01.012

IF_{2019} = 4,389

[4] E. Csapó, H. Szokolai, Á. Juhász, **N. Varga**, L. Janovák, I. Dékány, *Cross-linked and hydrophobized hyaluronic acid-based controlled drug release systems*, Carbohydrate Polymers, 195 (2018) 99-106. doi: 10.1016/j.carbpol.2018.04.073

IF_{2018} = 6,044

[5] Á. Juhász, **N. Varga**, Á. Turcsányi, E. Csapó, *Relation between Rheological, Structural and Dissolution Properties of Covalently and Ionically Modified Hyaluronic Acid-based Drug Carriers*, Conference Proceedings of 10th Anniversary International Conference on Nanomaterials - Research & Application, October 17th - 19th 2018, Brno, Czech Republic, pp.330-336, ISBN 978-80-87294-89-5.

Σ IF = 20,016

Other publications:

[6] A. N. Kovács, **N. Varga**, Á. Juhász, E. Csapó, *Serum protein-hyaluronic acid complex nanocarriers: structural characterisation and encapsulation possibilities*, Carbohydrate Polymers, 251 (2021) 117047. doi: 10.1016/j.carbpol.2020.117047

IF_{2019} = 7,182

[7] A. N. Kovács, **N. Varga**, Gy. Gombár, V. Hornok, E. Csapó, *Novel feasibilities for preparation of serum albumin-based core-shell nanoparticles in flow conditions*, Journal of Flow Chemistry, 10 (2020) 497-505. doi: 10.1007/s41981-020-00088-4

IF_{2019} = 3,622

[8] L. Mérai, **N. Varga**, Á. Deák, D. Sebők, I. Szenti, Á. Kukovecz, Z. Kónya, I. Dékány, L. Janovák, *Preparation of photocatalytic thin films with composition dependent wetting properties and self-healing ability*, Catalysis Today, 328 (2019) 85–90. doi: 10.1016/j.cattod.2018.10.015

IF_{2019} = 5,825

[9] B. Kutus, **N. Varga**, G. Peintler, A. Lupan, A. A. A. Attia, I. Pálinkó, P. Sipos, *Formation of mono- and binuclear neodymium(III)-gluconate complexes in aqueous solutions in the pH range 2-8*, Dalton Transactions 46 (2017) 6049-6058. doi: 10.1039/C7DT00909G

IF_{2017} = 4,099

[10] B. Kutus, D. Ozsvár, **N. Varga**, I. Pálinkó, P. Sipos, *ML and ML2 complex formation between Ca (II) and D-glucose derivatives in aqueous solutions*, Dalton Transactions 46 (2017) 1065-1074. doi: 10.1039/C6DT04356A

IF_{2017} = 4,099

Σ IF = 24,827

ΣΣ IF = 44,843

Oral and poster presentations related to the scientific topic of the dissertation:

1) Á. Turcsányi, **N. Varga**, V. Hornok, Á. Juhász, E. Csapó: *Encapsulation efficiency of chitosan, hyaluronan and chitosan/hyaluronan composite nanoparticles*, 9th International Colloids Conference, 16-19 June, 2019, Barcelona-Sitges (poster)

2) **N. Varga**, V. Hornok, L. Janovák, I. Dékány, E. Csapó: *Biocompatible PLA and PLGA nanoparticles: effect of synthesis conditions and tunable hydrophilicity on the drug encapsulation efficiency*, 9th International Colloids Conference, 16-19 June, 2019, Barcelona-Sitges (poster)

3) **Varga N.**, Csapó E.: *PLA és PLGA nanorészecskék szerkezetének és kapszulázási hatékonyságának vizsgálata*, Tavaszi Szél Konferencia 2019, május 3-5, 2019, Debrecen (oral)

4) **Varga N.**, Hornok V., Janovák L., Dékány I., Csapó E.: *PLA és PLGA nanorészecskék szerkezetének és kapszulázási hatékonyságának vizsgálata*, XLI. Kémiai Előadói Napok, 2018. október 15–17, Szeged (oral)

5) **N. Varga**, V. Hornok, J. Dusnoki, Á. Juhász, E. Csapó, I. Dékány: *Preparation and characterization of PLGA nanoparticles for drug delivery*, SIWAN8, 8th International Workshop on Advances in Nanoscience, 7-10 October, 2018, Szeged (poster)

6) Á. Juhász, **N. Varga**, H. Szokolai, E.Csapó: *Cross-linked and neutralized hyaluronic acid-based drug delivery systems*, 9th Global Chemistry Congress, 22-23 July, 2018, Lisbon (poster)

7) I. Dékány, E. Csapó, V. Hornok, Á. Juhász, Á. Deák, **N. Varga**, L. Janovák: *Self-assembled nanostructures for drug delivery: structural properties and thermodynamic state functions*, 11th Conference on Colloid Chemistry (11CCC), 28-30 May, 2018, Eger (oral)

Other presentations:

1) **Varga N.**, Juhász Á., Csapó E., *Polimer alapú kolloidális gyógyszerhordozók „kvázi" kétdimenziós szenzortechnikák általi jellemzése*, XLII. Kémiai Előadói Napok, 2019. október 28–30, Szeged (oral)

2) V. Varga, H. Szokolai, A. N. Kovács, **N. Varga**, E. Csapó, I. Dékány: *Investigation of different human serum albumin-based composites for kynurenic acid drug delivery*, Chemistry Physics and Biology of Colloids and Interfaces, 2-6 June, 2019, Eger (oral)

3) B. Kutus, **N. Varga**, G. Peintler, I. Pálinkó, P. Sipos, *Equilibria and structure of neodymium(III)-gluconate complexes forming in acidic to slightly basic medium*, YoungChem 2016, October 5-10, 2016, Czestochowa (oral)

4) **Varga N.**, Kutus B., Peintler G., Pálinkó I., Sipos P., *Neodímium-glükonát komplexek oldategyensúlyi és –szerkezeti jellemzése*, 50. Komplexkémiai Kollokvium, május 30. – június 1, 2016, Balatonvilágos (oral)